CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

DETECTION OF ADULTERATION IN RAW COW MILK SUPPLIED IN THE QAZVIN PROVINCE, IRAN, DURING (2015-2016)

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Article history:
Fill be filled by editor

Keywords:
Raw milk;
Adulteration;
Health;
Quality;
Iran.

ABSTRACT

Milk is the most appropriate source of food required for growth of infants and Children and for preservation of health in adults, It supplies nutrients like proteins, carbohydrates, fat, vitamins and minerals in moderate amounts in an easily digestible form. Improving milk quality; in addition to maintaining the health satisfaction of milk and its products consumer, is really important. Milk adulteration is a very common food fraud and is a big social problem both in the backward and advanced countries. Apart from the ethical and economical issue, it also causes serious health problems**.** So, given the importance of above facts, this study aims to detect the some of common adulteration was conducted in raw cow milk collected from Qazvin province. A total 61 raw milk samples were collected from 15 collection center of raw milk during the different seasons (2015-2016). The results showed that, 4.9% of the milk samples were adulterated with water. Formalin, hydrogen peroxide and salt were detected as 16.4, 11.5 and 34.4 % in the milk samples, respectively. None of the samples were contaminated with bicarbonate. The number of samples positive containing salt, formalin, hydrogen peroxide, added water and bicarbonate were as 21, 10, 7, 3 and 0, respectively. Positive cases of formalin, salt and hydrogen peroxide in the first six months were higher than the second six months. However, the only positive cases of formalin was significant between warm and cold seasons ($P<0.05$)**.** Milk used for human consumption can be adulterated with cheaper materials or hazardous chemicals. Thus, more analysis is essential to create awareness among the consumers regarding malpractices and negligence in milk production, especially in the warm seasons.

1.Introduction

Milk is a complex compound made up of protein in colloidal suspension form, fat emulsion and a lactose in a actual solution, it also contains vitamins, enzymes minerals and organic compounds such as lactic and citric acids. Therefore, milk is almost a complete food that can be providing lots of human nutritional needs (Harding, 2003; Karim, 2008).

Milk is one of the most consumed dairy products, with high nutritional value. Thus, its use is recommended for all age groups (Harding, 2003; Karim, 2008). Milk is needed for growth and maintenance of health, However it can be used as a vehicle for the transmission of chemicals and other impurities (Nirwal et al., 2013). According to definition provided by the International Milk Commission, raw milk delivered to the factory must be fresh, pure, clean, and free of colostrum and with natural taste and smell. Also Milk should be nothing added or taken from it (including extracting fat, adding water, salt, sugar, dried milk, baking soda, hydrogen peroxide and formalin

(Barbano et al., 2006). Milk can primarily contaminate with microbial or chemical agents(drugs, mycotoxins, preservatives, insecticide, and etc.). It can also contaminate secondarily with milking equipment, employees, and various processes and at the stage of transition to the factory. On the other hand, there is a variety of adulterations in milk that their recognition is also useful for consumer (Tipu et al., 2007).

Milk adulteration is a very common food fraud and is a big social problem both in the backward and advanced countries. Apart from the ethical and economical issue, it also causes serious health problems associated with the milk health, subsequently health of consumers which are added to milk in order to lower microbial load and hide defects (Barbano et al., 2006; Das et al., 2016). Sometimes milk is adulterated with low value Ingredient like water or whey for the purpose of increasing the profits and reducing the cost of its production. Adding water or 'liquid-whey' to milk is a very common practice by the milk supplier to increase its volume. Diluted milk reduces its nutritional value. Also, if the water is contaminated, for example, with chemicals or pathogens, it causes serious health problems in consumers. In addition to, added water changes specific gravity of the milk and its natural color gets destroyed. To compensate specific gravity by the milk supplier's different types of salt and sugar is added to milk which in turn can be dangerous for the consumer. Sometimes to maintain the natural color of milk a small amount of coloring matter is added (Das et al., 2016). Other the common adulteration include in starch, chlorine, hydrated lime, sodium carbonate, formalin, hydrogen peroxide, ammonium sulfate, urea, boric acid and various antibiotics which are used for different purposes (Das et al., 2016; Tipu et al., 2007).For example, neutralizers like sodium hydroxide, sodium carbonate or sodium bicarbonate to neutralize the acidity of the milk are added so that the consistency and shelf life of the milk is increased. Hydrogen peroxide is added to milk to lengthen its freshness. It can damage the digestive system cells and lead to gastritis and inflammation of the intestine. Urea is added to adjust the solids-non- fat of milk, to provide whiteness and giving actual appearance to milk.it causes pain in lower abdomen, muscle cramps, irregular heartbeat, numbness and weakness in hands and feet, chills and shivering fever (Singuluri and Sukumaran 2014). Some of the common adulteration, the aim of adding them and their effects on human health is shown in table1 (Afzal et al., 2011; Ramya et al., 2015). Keeping in view the above facts, determination of milk adulteration is very important.

Therefore, the present study was conducted to detect some of the common adulteration using standard methods in raw cow milk samples collected from raw milk collection centers in Qazvin province of Iran.

Table1. Some of the common adulteration, the aim of adding them and their effects on human health (Barbano et al., 2006; Das et al., 2016; Tipu et al., 2007)

Types of adulteration	Goal of addition	Effects on human health
Hydrogen peroxide	Slow down microbial growth and delay spoilage of milk	The effect on the digestive system cells and creating gastroenteritis
Formalin	As microbial growth inhibitor is added to the milk	Causes diarrhea, vomiting and abdominal pain. in high doses may cause decreased the body temperature, weak irregular pulse, shallow respiration, ,unconscious and blindness
Bicarbonate	neutralize the high acidity of milk	Cause disruption in the activity of growth hormones and reproduction

Salt	Hiding added water to the milk, set the density and freezing point of milk	Taking too much salt increases the risk of cardiovascular diseases
Added water	Increase the volume of milk	Decrease the nutritive value of milk and following that nutritional disorders in humans. If contaminated poses a health risk especially for sensitive people.
Urea	To adjust the solids-non- fat of milk, to provide whiteness and giving actual appearance to milk	causes pain in lower abdomen, muscle cramps, irregular heart beat, numbness and weakness in hands and feet, chills and shivering fever
Starch	To adjust the density of diluted milk	It can cause diarrhea. Can be dangerous for diabetic patients.

2. Materials and methods

2.1. Samples Collection

A total of 61 samples of raw milk from raw milk collection centers mainly on Qazvin city during four seasons (The number of samples in each of the season's autumn, winter and summer 15 and in spring 16) were collected. The samples transported under appropriate conditions to Health and Food Safety Laboratory of Qazvin University of Medical Sciences and Some common Adulteration (including added water, salt, bicarbonate, formalin and hydrogen peroxide) were analyzed in the samples collected.

2.2. Detection of Adulterations

2.2.1. Water detection

Added water Adulterations was determined through specific gravity using Thermolac-densimete (Mahmoudi and Norian, 2015).

2.2.2. Sodium chloride detection

To detect salt, 5 ml of silver nitrate 34.1 g/ l was mixed with a few drops of 5% potassium dichromate and then 1 ml of milk were added to it. Appearance of yellow color indicates presence of sodium chloride (Tipu et al., 2007).

2.2.3. Hydrogen peroxide detection

For determining Hydrogen peroxide 5 ml of milk were poured in a test tube and 5 drops of a solution of 2% para-phenylenediamine were added to it, then was stirred well. The blue color indicates the presence of hydrogen peroxide in milk (Tipu et al., 2007).

2.2.4. Bicarbonate detection

For bicarbonate 10 ml milk samples were taken in test tube and 10 ml 95 % ethyl alcohol were added. Five drops of rosolic acid (1% ethanol) were added and mixed well. Pink color indicates presence of carbonate (Tipu et al., 2007).

2.2.5. Formalin detection

Formalin was detected by the method hehner's test (Tipu et al., 2007).

2.3. Statistical Analysis

All the experiments were performed in triplicate. Statistical analysis was analyzed in SPSS-19 using Chi-square test at significance level $P<0.05$.

3. Results and discussions

The results of this study (Table 2) showed that respectively 34.4% and 4.9% of the milk samples had salt and extra water. Formalin and hydrogen peroxide adulteration was present in 16.4% and 11.5% samples. No sample was found to be adulterated with bicarbonate**.** Positive cases of salt and hydrogen peroxide in the first six months were higher than the second six months (Figure 1). However, only positive cases of formalin were significant between warm and cold seasons ($P<0.05$).

Milk in its natural form is one of the most important and completes natural food that is consumed by people of all ages as various dairy products. Given the properties of milk, control of this valuable product and measuring its

ingredients are completely essential for milk producers and the dairy industry. Because, milk quality has effect directly on milk processing and quality of dairy products (Nirwal et al., 2013).

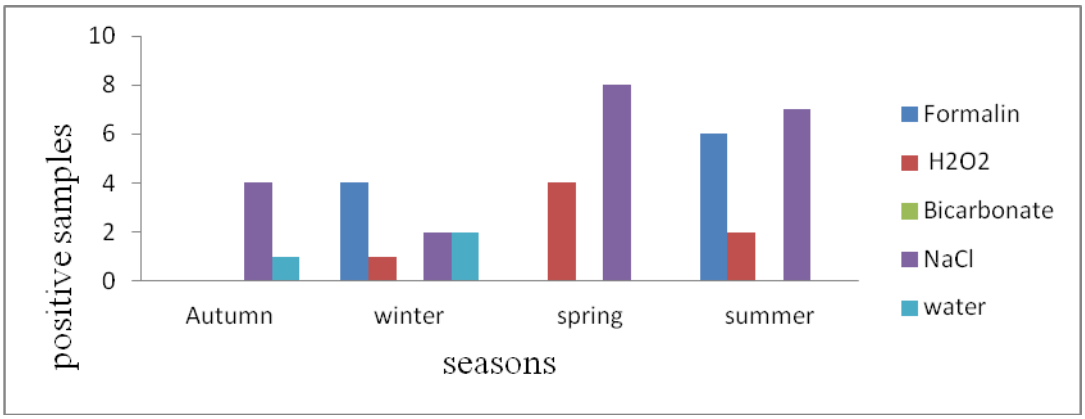

Figure 1. Comparing the Adulteration in milk samples during difference seasons

Table 2. Presence of adulterant (% positive sample) in the raw cow milk collected from Qazvin province (2015-2016)

		Various Adulteration				
seasons	**Sample size (n)**	**Formalin (%)***	**hydrogen peroxide (%)**	**salt (%)**	**Bicarbonate (%)**	**Added water (%)**
Autumn	15	0(0%)	0(0%)	4(26.7%)	0(0%)	1(6.7%)
Winter	15	4(26.7%)	1(6.7%)	2(13.3%)	0(0%)	2(13.3%)
spring	16	0(0%)	4(25%)	8(50%)	0(0%)	0(0%)
summer	15	6(40%)	2(13.3%)	7(46.7%)	0(0%)	0(0%)
Total	61	10(16.4%)	7(11.5%)	21(34.4%)	0(0%)	3(4.9%)

*There was significant difference between cold and warm seasons ($P<0.05$).

Table 3. The percentage of some adulteration in several countries

Country	**Type of sample**	***Various Adulteration***							
		(N)	**Formalin**	**H_2O_2**	**salt**	**Neutralizing**	**water**	**urea**	**Reference**
India	Raw milk	50	6(12%)	13(26%)	27(54%)	_	_	12(24%)	(Ramya et al., 2015)
India	Raw milk	50	16(32%)	16(32%)	41(82%)	13(26%)	_	30(60%)	(Singuluri and Sukumaran 2014)
Iran	Raw milk	60	0(0%)	0(0%)	6(10%)	7(11.6%)	24(40%)	_	(Mahmoudi et al., 2015)

Iran	Raw milk	112	0(0%)	2(1.74%)	0(0%)	0(0%)	18(16.6%)	_	(Mahmoudi and Norian, 2015)
Sudan	Raw milk	240	5(2.08%)	1(0.41%)	_	_	_	_	(El Zubeir and El Owni, 2009)
Pakistan	Pasteurized	60	(27%)	(3%)	_	_	(96%)	(86%)	(Faraz et al., 2013)
Pakistan	Milk	100	11(11%)	9(9%)	8(8%)	11(11%)	74(74%)	7(7%)	(Barham et al., 2015)
Bangladesh	Raw milk	50	5(10%)	0(0%)	_	10(20%)	50(100%)	_	(Chanda et al., 2013)
Brazil	UHT milk	100	(44%)	(30%)	_	_	_	_	(Souza et al., 2011)

On the other hand, environmental factors and livestock characteristics affect the quality of raw milk. Thus, maintaining quality of raw milk in order to maintain its competitive place in the market is critical and any change on its composition is serious threat to producers of milk (price cuts), dairy industry (production costs), and consumers (dietary and health aspects (Smit et al., 2000). At the time of buying milk, consumers have right to receive healthy and adulteration -free milk. Adulteration of milk may be intentional or unintentional occur in the process of production. The first type is intended to increase the margin of profit and second type of adulteration may be incidental contamination which is usually due to ignorance, negligence or lack of proper facilities (Kamthania et al., 2014; Nirwal et al., 2013). Consumers are often become victim of diseases by consuming adulterated milk. Diseases of the kidneys, skin, eye, heart problems and even cancer are some of the common disease caused by consuming adulterated milk (Das et al., 2016). For example, adding of carbonate in milk causes digestive problems such as stomach ulcer, diarrhea, colon cancer and Impaired in the balance of body fluids. Hydrogen peroxide with disruption in the operation of body's natural antioxidant system causes early aging. Chlorides in milk also causes imbalance in blood acid, base, and pH (Mahmoudi and Norian, 2015). So, the aim of this study was to determine and assess adulteration in raw cow milk supplied in the Qazvin province. Results obtained from this study showed that numbers of positive raw milk samples for formalin, hydrogen peroxide and salt in the warm seasons (Spring and summer)is higher in compared to the cold seasons(autumn and winter). In the warm seasons, due to high temperature and difficulties in Maintenance of milk under standard conditions the possibility addition of neutralizing materials and microbial growth inhibitors to hide the acidity and spoilage of milk is more. Assessment of raw milk adulteration in Bangladesh showed that 100% of the milk samples had added water and 20% and 10% of milk samples was adulterated with sodium bicarbonate and formalin, respectively. The results of the study above showed that, there is a positive relationship between temperature and the amount of adulteration. So that, the numbers of positive cases containing formalin and bicarbonate were more in the warm months compared to cold months that its consistent with our results (Chanda et al., 2013).

The results of the assessment of physicochemical properties and adulteration in the samples of raw cow milk collected from 14 semi-industrial dairy farms across Province in Qazvin, Iran during 2011 showed that respectively 1.78% and 0.89%of milk samples were contaminated with hydrogen peroxide and hypochloride. Added water was also16.7%. In mentioned study all samples in terms of formalin, carbonate and salt were negative (Mahmoudi and Norian, 2015). Density of raw milk is in the range of 1.028- 1.034 kg/m3 at

15°C. This means that weight of a liter of milk at 15°C is between 1028 to 1034 grams. Taking fat of milk increases the specific gravity of milk. Conversely, added water reduces the specific gravity of milk. In this study specific gravity 4.9% of the samples were lower than the normal range that was being related to the cold seasons. But, the specific gravity of milk was no significant difference in cold and warm seasons (Karim, 2008).The survey of 240 raw cow milk samples in Sudan (2009) showed that 5 samples were contaminated with formaldehyde and 1 sample was contaminated with hydrogen peroxide (El Zubeir and El Owni, 2009). Results of a study by Singuluri and Sukumaran (2014) in India on 50 raw cow milk samples showed that 41(82%), 16(32%) and 13(26%) of samples were contaminated with salt, hydrogen peroxide and neutralizing, respectively (Singuluri and Sukumaran 2014).

Investigation of adulteration in raw cow milk samples collected from East Azerbaijan Province of Iran showed that of the 60 samples collected, 7(11.6%) and 6(10%) of samples were contaminated with Carbonate and salt respectively. Also, 24(40) % of samples had Added water. But formalin and hydrogen peroxide was negative in all samples (Mahmoudi et al., 2015).

A study conducted by Ramya et al (2015) showed that 12 percent of the samples were contaminated with formalin and 26 percent with hydrogen peroxide. Also 5% of the samples were containing sodium chloride (Ramya et al., 2015). In the present study the extent of adulteration with formalin is 16.4%, which was close to the results mentioned study.

Results of a study by Faraz et al. in Pakistan on 60 milk samples collected from canteens of educational institutes and public places showed that 97% of samples had added water. Urea, formalin and hydrogen peroxide were detected as 87%, 27% and 3% of samples, respectively (Faraz et al., 2013). The percentage of some adulteration in several countries is shown in table 3.

In comparison with the above mentioned studies, water adulteration in this study was lower. In contrast, adulteration percent of formalin in the current study was more than aforementioned studies. However, the case of contamination with hydrogen peroxide (11.5%) was also remarkable.

4. Conclusions

The obtained data from this study and previous studies indicate that milk adulterations are quite common in many regions worldwide developing countries or backward countries. Adulteration of milk may be intentional or unintentional occur in the process of production. To prevent milk adulteration, providing training and necessary instructions is essential for the distributors of milk and animal breeders. Milk is an essential food for humans, especially for children, pregnant women and patients. Therefore, consumption of lower quality milk can create serious health problems in human. At the time of buying milk, consumers have right to receive healthy and adulteration -free milk.so, it seems that the only remedy to this problem is to create awareness among consumers about their rights.

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Acknowledgement

This work was financially supported by Qazvin University of Medical Sciences, Qazvin,Iran.